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Zoological Research

GENETIC DIVERSITY IN THE WILD POPULATION AND HATCHERY STOCK OF *Penaeus japonicus* SHRIMP BY ISOENZYME ANALYSIS

日本对虾野生种群和养殖群体的同功酶遗传变异

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Penaeid shrimp *Penaeus japonicus*, commonly named as Flowery Shrimp or Banded Shrimp, is one of the most commercially important species of marine crustacean fishery in China. Being of the warm-water species conducting short distance migration, the animal is mainly distributed in the coastal waters of south China, i.e. the southern part of the East China Sea and the South China Sea (Liu *et al.*, 1986). Since 1992 after shrimp epizootic attacked the seafarming industry of *Penaeus chinensis*, the mariculture and transplant of *P. japonicus* from the southern to northern seawaters of China has successfully been practiced (Zhu *et al.*, 1998; Su *et al.*, 1996). As a result, *P. japonicus* is regarded as one of the most promising species not only enriching the mariculture categories but also enabling to build a new stock by hatchery release in the north seawaters of China. But, the relevant activities like shrimp husbandry and large-scale release in the non-native waters were performed in lack for necessary comprehension to the genetic background. However, many instances of genetic diversity decrease induced by mariculture and sea ranching have been documented (Crozier, 1993; Sbordoni *et al.*, 1986; Taniguchi *et al.*, 1983).

Electrophoresis has been applied to evaluate genetic diversity in interspecies and intraspecies of Penaeid shrimps (Garcia *et al.*, 1994; Harris *et al.*, 1990; Lester, 1983; Mulley *et al.*, 1980, 1981a, b; Sunden *et al.*, 1991; Tam *et al.*, 1993). To obtain the initial results of isoenzyme variation in the wild population of *P. japonicus* from the native waters and the cultivated stock in the north China, the vertical slab polyacrylamide-gel electrophoresis was employed.

1 Materials and Methods

1.1 Mariculture strategy for *P. japonicus* in the north China

Usually, the post-larvae of *P. japonicus* for seafarming in the northern China are produced by the hatchery stations in the southern China or by the local hatchery stations along the sea coast of Shandong, Hebei and Liaoning Provinces, as well as Tianjin. Some of the local hatcheries develop nauplii bred by the south hatcheries and some directly introduce the wild adults of *P. japonicus* captured in those waters south to the Taiwan Strait, i.e. the coastal waters of Fujian and Guangdong Provinces. But, some hatcheries use the grown-out offspring after indoor wintering as a broodstock for hatchery-produced larvae. Most of these hatchery stations produce post larvae from May to September to meet the requirements of most shrimp farms conducting two crops of harvest within one year.

1.2 Experiment samples

1.2.1 The wild population: represented by 30 individuals (215 mm in mean body length) of 400 adults captured in the waters of the Taiwan Strait (in the vicinity of 23°00'N, 120°30'E) in April 1998. The index samples were collected in May 1998 after spawning. The hatchery production from this broodstock was performed in the Laoshan Tuzhai Shrimp Hatchery Station near Qingdao.

1.2.2 The hatchery stock: represented by 30 grown-out individuals (110 mm in average body length), reared in the earthen pond of the Laoshan Tuzhai Shrimp Hatchery Station. The index samples were obtained in August 1998 after about 80-day growing-out. The hatchery stock was presumed as the offspring of the above-mentioned broodstock.

1.3 Sample preparation

The live animals, 30 individuals of each population, were collected and transported to the Lab. The muscle and liver tissues dissected from each live shrimp were kept in liquid nitrogen as the material source. 0.2 - 0.3 g sub-sample of tissue was homogenized in 5 volume 0.1 mol/L PBS with ice-bath. Homogenates of muscle and liver were then centrifuged at 4℃, at 15 000 r/min for 10 min to gain supernatant. Added morsel of 1% tetrabromophenol sulfonphthalein to the obtained supernatant and mixed with isometric 40% saccharose solution. The supernatant was used as the enzyme source and analyzed by electrophoresis within one day.

1.4 Electrophoresis and zymogram analysis

Applying 7.5% polyacrylamide-gel and Tris-Glycin (TG) or Tris-Citrate (TC) electrode buffer (Table 1), electrophoresis

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was conducted at 4℃ for 5 h at 350 constant voltage. Histochemically staining gels was performed subject to the protocol in "Experimental Methods and Techniques for Genetics" and "Handbook of Enzyme Electrophoresis in Human Genetics" (Wu *et al*., 1983; Harris *et al*., 1976) with a few modifications. After electrophoresis, rinse the stained gels with double distilled water and soak them in 7% acetic acid to mount. The enzymograms were recorded by photographing. The experiment totally investigated 20 enzymes, seven of which (alanopine dehydrogenase, aspartate aminotransferase, creatine kinase, formaldehyde dehydrogenase, glutamate-ammonia-ligase, glucose dehydrogenase and succinate dehydrogenase) were unscoreable probably due to their extremely low activities. With banding patterns, twelve enzymes were used to estimate the genetic diversity except Esterase due to its complex pattern (Table 1).

Table 1 Enzymes analyzed and the buffer system applied

Enzymes	E.C.No	Substructure	Tissues	Buffer
LDH	1.1.1.27	Tetramer	Muscle	TG
MDH	1.1.1.37	Dimer	Muscle	TG
G6PD	1.1.1.49	Dimer	Muscle	TG
ADH	1.1.1.1	Dimer	Liver	TG
ME	1.1.1.40	Tetramer	Muscle	TC
GPI	5.3.1.9	Dimer	Muscle	TG
AK	2.7.4.3	Monomer	Muscle	TG
IDH	1.1.1.42	Dimer	Muscle	TC
ACP	3.1.3.2	Monomer	Liver	TG
AKP/ALP	3.1.3.1	Monomer	Liver	TG
SOD	1.15.1.1	Dimer	Liver	TG
GDH	1.4.1.2	Tetramer	Liver	TG
EST	Unspecific	Unspecific	Liver	TG

LDH: Lactate dehydrogenase; MDH: Malate dehydrogenase; G6PD: Glucose-6-phosphate dehydrogenase; ADH: Alcohol dehydrogenase; ME: Malic enzyme; GPI: Glucosephosphate isomerase; AK: Adenylate kinase; IDH: Isocitrate dehydrogenase; ACP: Acid phosphatase; AKP/ALP: Alkaline phosphatase; SOD: Superoxide dismutase; GDH: Glutamate dehydrogenase; EST: Esterase.

The nomination of loci and description of alleles were conducted according to Shaklee's method (Shaklee *et al*., 1989). To assess the genetic structures of these two populations, proportion of polymorphic loci (P), effective number of alleles per locus (N_e), allelic frequency, observed heterozygosity (H_0), expected heterozygosity (H_e) and divergent index (D) at Hardy-Weinberg equilibrium were calculated. The genetic similarity (I) and genetic distance (d) were also applied to estimate the enzyme variation between these two populations.

2 Results

Of 22 loci encoding 12 enzymes, four polymorphic loci (sMDH, ACP-1, AKP-2 and GDH-1) were detected in the index samples of the wild population and one polymorphic locus (SOD-1) was detected in the index samples of the hatchery stock. Figure 1 exemplifies the polymorphisms of AKP-2 detected in the wild population whilst the hatchery stock showed monomorphism. As listed in Table 2, it is explicit that the gene distribution characterized in isozygoty for the most common allele-

Table 2 Gene frequency in the wild population and hatchery stock of *P. japonicus*

Locus	Allele	Gene frequency	
		Wild population	Hatchery stock
LDH	100	1.0	1.0
SMDH	40	0.017	0.0
	100	0.983	1.0
MMDH	100	1.0	1.0
G6PDH	100	1.0	1.0
ADH	100	1.0	1.0
ME	100	1.0	1.0
IDH-1	100	1.0	1.0
IDH-2	100	1.0	1.0
GPI	100	1.0	1.0
AK-1	100	1 0	1.0
AK-2	100	1.0	1.0
ACP-1	100	0.967	1.0
	92	0.033	0.0
ACP-2	100	1.0	1.0
AKP-1	100	1.0	1.0
AKP-2	100	0.967	1.0
	88	0.033	0.0
AKP-3	100	1.0	1.0
SOD-1	100	1.0	0.917
	110	0.0	0.083
SOD-2	100	1.0	1.0
SOD-3	100	0.0	1.0
GDH-1	100	0.983	1.0
	120	0.017	0.0
GDH-2	100	1.0	1.0
GDH-3	100	1.0	1.0

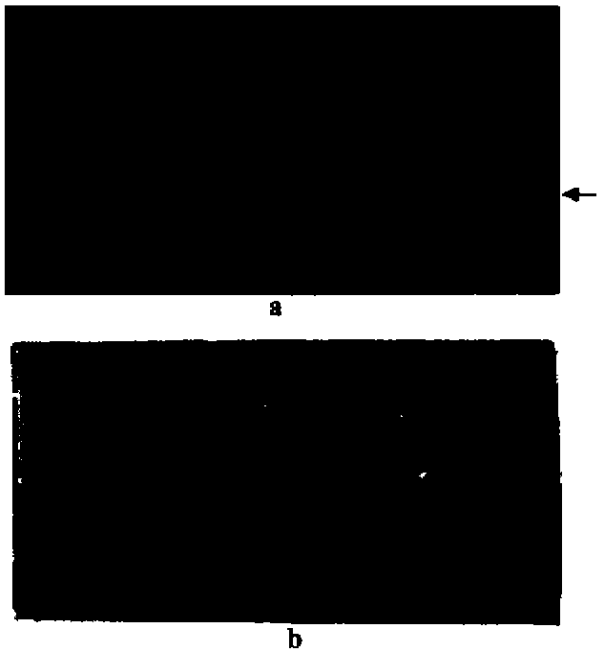

Fig. 1 Electrophogram of AKP in *Penaeus japonicus*
a. Wild population; Arrow indicates the polymorphic loci detected;
b. Hatchery stock.

les possessed a percentage above 0.90. Based on the genotypic distributions, the clear extinction manifestation of the rare isozygoty allelic genotypes existed in all loci, which implies that the heredity of the rare alleles was mostly performed by heterozygotes rather than by homozygotes. The allelic frequency in two populations is demonstrably diverse.

Accounting a locus polymorphic if the frequency of the most common allele was 0.99 or less, the average proportion of polymorphic loci (P) was 18.2% for the wild population and 4.5% for the hatchery stock. The observed and expected heterozygosities (H_o and H_e) of the hatchery stock were 0.0015 and 0.0069, respectively. And the divergent index (D) valued −0.781, which implies a critical deficit of heterozygotes on the assumption of Hardy-Weinberg equilibrium. While, the observed and expected heterozygosities of the wild population were 0.0091 (H_o) and 0.0088 (H_e), respectively; and the corresponding D value was 0.021, indicating a slightly excess heterozygotes. The Ne (number of effective alleles per locus) values of the wild and hatchery populations were 1.05 and 1.03 respectively. The genetic similarity (I) between the two populations was 0.98 and their genetic distance (d) was 0.020 (t = 21.84). Table 3 summarizes the average estimates of genetic diversity in these two investigated populations in terms of the electrophoretic data.

Table 3 Mean estimates of genetic diversity at 22 enzyme loci in the index samples of *P. japonicus*

Population	P	H_o	H_e	D
Wild	18.2	0.0091	0.0088	0.021
Hatchery	4.5	0.0015	0.0069	−0.781
Population	N_e	d	I	
Wild	1.05	0.020	0.98	
Hatchery	1.03			

3 Discussion

This study primarily reported the genetic variability at 22 enzyme loci in *P. japonicus* populations bred in the north China where is of non-native waters for this species so as to enrich apprehension to genetic structure of marine valuable animals. The obtained results are fairly accordant with those previously reported electrophoresis investigations in Penaeid shrimp with polymorphism levels varying from 11% − 32% (Harris *et al.*, 1990; Mulley *et al.*, 1980; Lester, 1983; Sbordoni *et al.*, 1986; Sunden *et al.*, 1991). Recently, Wang (1999) studied on the genetic structure in the two populations of *P. chinensis* distributed in the Yellow and Bohai Seas and revealed a 15% − 20% polymorphism. The relatively low levels of polymorphism are very ubiquitous in most Penaeid shrimp species due to fewer alloezymes scored.

The heterozygosity (0.0091) observed in the wild population ranks in the inferior limit ranging from 0.006 to 0.09 for other Penaeid shrimp. But it is much far less than the mean value of 0.073 in crustaceans and the average value of 0.051 in Penaeoidea and Caridea (Hedgecock *et al.*, 1982). In comparison with the data reported by Sbordoni *et al.* (1986), both polymorphism and heterozygosity in the wild population of *P. japonicus* from the Taiwan Strait are relatively lower than that in two wild broodstocks introduced from Japanese waters to Italy (Table 4). This lower level of genetic diversity may be correlated with the smaller stock size suffered from overexploitation and effected by those individuals escaped from the adjacent mariculture farms, which have been operating for two decades on the two littoral sides of the Strait. From this point of view, the germplasm resources of *P. japonicus* could not be optimistic although the divergent index D valued 0.021 indicating a slightly excess of heterozygotes on the assumption of Hardy-Weinberg equilibrium in this study.

Some evidences have been reported in genetic effects induced by aquaculturing and seafarming, where fishery husbandry and large-scale releases make considerable reduction of genetic diversity. Human fishery activities often make intentional or unintentional selecting for some particular traits that may not be best suited to long-term environmental variability. And the decrease of genetic diversity is normally characterized by loss of rare alleles and changes of allelic frequencies. As Sbordoni *et al.* (1986) reported, the mean heterozygosity has been reduced by 60% after successive culturing for several generations. In this study, four rare alleles (sMDH^{40}, ACP-1^{92}, AKP-2^{88} and GDH-1^{120}) stood for loss in the index samples of the hatchery population. The minus D value (−0.781) indicates a critical deficit of heterozygotes. The main causes of the distinct decrease of polymorphism and heterozygosity were mainly of genetic bottleneck and drift in the hatchery population as well as inbreeding. Regarding the phenomenon that a polymorphic locus (SOD-1) was scored in one individual of the index hatchery samples, this may be partially due to sampling randomness and partially due to enzyme activity induced by environmental factors like changes of feeding categories and habitat.

The shortage of wild broodstock for *P. japonicus* mariculture in the northern China not only further stimulates the development of hatchery technology and broodstock husbandry but also promotes establishment and application of recycling broodstock. Because of considerably high fecundity but flexible fertility obtained by artificially maturing mostly through unilateral eyestalk extirpating, hatched larvae may often come from a very limited amount of parents that perform successful ovulating (You *et al.*, 1998). Under these conditions, the random genetic drift and inbreeding, to some extent, exist in most successive aquaculture systems. In return, the unfavorable changes of gene pool of hatchery population probably result in a significant decrease in the relevant features like the adaptability to environment, growth rate and disease resistance. This may be one of the main reasons why the shrimp epizootic has been overwhel-

Table 4 Estimates for three wild populations of *P. japonicus*

Population	P	H_o	H_e
Wild-T	18.2	0.0091	0.0088
Wild-K	35.0	0.1020	0.1050
Wild-S	20.0	0.0640	0.0720
Population	H_o/H_e	N_e	Sample location
Wild-T	1.034	1.05	Taiwan Strait
Wild-K	0.971	1.40	Kyushu coastal waters
Wild-S	0.889	1.30	Seto Inland Sea

ming and often causes a very poor harvest in the shrimp farming industry. Of particular concern is the large scale hatchery releasing for restocking or enhancing the recruitment have been implemented in lack for essentially assessing and monitoring the genetic effect. A management mechanism for shrimp enhancement should be vitally urged. At least, to stop using those shrimp seedlings produced from a successive aquaculture system as the source of hatchery release is highly recommended so as to sustain the genetic diversity of the releasing stock to the reliable or utmost extent. Besides, further studies on the genetic structure of other *P. japonicus* geographic populations distributed in China Waters will be of significance and interest to a selective breeding program.

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